TRADE SECRET

Study Title

H-28072: Static, Acute, 96-Hour Limit Test with Rainbow Trout, *Oncorhynchus mykiss*

TEST GUIDELINES: OECD Guideline for the Testing of Chemicals
Section 2 (Part 203) (1992)

AUTHOR: Barbra D. Ferrell, B.S.

ORIGINAL REPORT COMPLETED ON: December 10, 2007

REPORT REVISION 1 COMPLETED: July 14, 2008

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LABORATORY PROJECT ID: DuPont-22830

WORK REQUEST NUMBER: 17199

SERVICE CODE NUMBER: 228

SPONSOR: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current OECD and MAFF (Japan) Good Laboratory Practice.

Study Director: Barbra D. Ferrell 14 July 2008

Barbra D. Ferrell, B.S.
Associate Scientist

Date

QUALITY ASSURANCE STATEMENT

Work Request Number: 17199
Service Code Number: 228

Key inspections for DuPont work request 17199, service code 228 were performed for the tasks completed at DuPont by the Quality Assurance Unit of DuPont and the findings were submitted on the following dates.

Phase Audited	***Audit Dates***	***Date Reported to Study Director***	***Date Reported to Management***
Protocol:	May 30, 2007	May 30, 2007	May 30, 2007
Conduct:	June 4, 2007	June 4, 2007	June 4, 2007
Report/Records:	November 29-30, 2007	November 30, 2007	December 4, 2007
Report Revision 1:	July 8, 2008	July 8, 2008	July 8, 2008

Reported by: Donna M. Johnston 11 July 2008
Donna M. Johnston
Quality Assurance Auditor
Date

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Reviewed by: Robert A. Hoke — 9 July 2008

Robert A. Hoke, Ph.D.
Principal Research Ecotoxicologist and Manager

Date

Issued by Study Director: Barbra D. Ferrell — 14 July 2008

Barbra D. Ferrell, B.S.
Associate Scientist

Date

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STUDY INFORMATION

Substance Tested:
- HFPO Dimer Acid Ammonium Salt
- 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid, ammonium salt
- 62037-80-3 (CAS Number)
- H-28072

Haskell Number: 28072

Composition:

82.6%	Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionate*
13.9%	Water
3.5%	Ammonium
0.41%	Organic Impurities

* Note: The Ammonium-2,3,3,3-tetrafuoro-2-(heptafluoropropoxy) propionate component (HFPO Dimer ammonium salt) contains 0.1 ppm HFPO trimer ammonium salt.

Purity: See composition, above

Physical Characteristics: Clear and colorless concentrated aqueous solution

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

Study Initiated/Completed: May 30, 2007 / (see report cover page)

Experimental Start/Termination: June 4, 2007 / June 8, 2007

REASON FOR REVISION 1

Consistent reporting of endpoints across studies, based on guidance contained in OECD TG 201, 202 and 203.

SUMMARY

The acute toxicity of H-28072 to unfed fingerling rainbow trout, *Oncorhynchus mykiss* was determined in an unaerated, static, acute, 96-hour limit test. The test was conducted in accordance with the OECD Guideline for the Testing of Chemicals: 203.

The study was conducted with a 120 mg/L nominal limit concentration of H-28072 and a dilution water control at a mean temperature of 11.8°C (range of 11.7-12.4°C). The mean, measured H-28072 limit test concentration was 96.9 mg/L. The mean, measured limit test concentration was 80-120% of the nominal limit test concentration for the study. A single dilution water control test chamber and 3 replicate limit test concentration chambers with 10 fish in each chamber were used for testing (total of 10 fish in the dilution water control and 30 fish in the limit test concentration). Fish in the dilution water control ranged from 4.3 to 4.9 cm in standard length (mean 4.47 cm), and 0.941 to 1.493 g in wet weight, blotted dry (mean 1.068 g) at test end. Control loading at test end was 0.534 g/L.

No mortality was seen at the nominal 120 mg/L (96.9 mg/L mean, measured) limit test concentration of H-28072 or in the control at the end of the 96-hour limit test. The 96-hour LC_{50}, based on the nominal limit test concentration of H-28072, was greater than 120 mg/L.

The results are summarized as follows:

Nominal concentration of H-28072, mg/L[a]	dilution water control and 120
Mean, measured concentration of H-28072, mg/L	ND[b] and 96.9
96-hour LC_{50} for H-28072, based on nominal concentration, mg/L	greater than 120

a Not adjusted for 82.6% purity by analysis during preparation.
b ND denotes none detected at or above the limit of detection of 0.0001µg/L.

INTRODUCTION

The objective of this study was to assess the acute toxicity of H-28072 to unfed fingerling rainbow trout, *Oncorhynchus mykiss*, during an unaerated, static, acute, 96-hour limit test.

MATERIALS AND METHODS

A. Test Guidelines

The study design complied with the following test guidelines:

- OECD, Section 2 (Part 203): Effects on Biotic Systems, *Guideline for the Testing of Chemicals* (1992).

B. Test Substance

The test substance, H-28072, was supplied by the sponsor. The test substance contains 82.6% H-28072 by analysis (Appendix A).

The solubility and stability of H-28072 in HLWW at approximately 12°C was demonstrated under test conditions and is shown by the analytical recoveries.

C. Test Solution Preparation

The limit test concentration replicate test solutions were prepared by direct addition of H-28072 to DuPont Haskell Well Water (HLWW). For each replicate, a 120 mg/L test solution was prepared by adding approximately 2.4 g of test material to HLWW in 26 L stainless steel tank (final volume 20 L), and stirring for approximately 30 minutes. The test solutions were clear and colorless with no visible precipitate.

D. Dilution Water

Dilution water originated from the DuPont Haskell well which is 378-feet deep and is cased and sealed to bedrock. The hardness of the well water is adjusted to approximately 100-140 mg/L as $CaCO_3$ by the flow-proportioned addition of $CaCl_2$. The well water is then aerated, passed through a green sand filter to remove iron, and filtered through 50-, 10-, and 0.45-µm filters to remove particulates. The water is heated or chilled as appropriate and distributed through aged polyvinyl chloride piping. The dilution water is analyzed twice yearly for major anions and cations, metals, total organochlorine and organophosphate pesticides, and polychlorinated biphenyls (Table 1). The dilution water meets OECD[(1)] criteria and specifications.

E. Test Organism Culture

Rainbow trout, *Oncorhynchus mykiss*, were purchased from Thomas Fish Company, Anderson, California. The fish were held in a 272-L, circular, fiberglass holding tank (84-cm diameter, 49-cm deep) for 25 days in continuously-flowing well water at DuPont Haskell. During the

7-day period preceding the test, water temperatures ranged from 12.3 to 12.6°C (mean 12.5°C). The fish were fed freshly-hatched, live brine shrimp (San Francisco Bay Brand, Newark, California) and AquaMax® Starter Fingerling 300 5D03 (PMI® Nutrition International, LLC) three times weekly. Sickness, injury, and visible abnormalities were not present in fish in the holding tank nor in the fish used for testing. Mortality did not exceed 1% during the 48 hours prior to use for testing. Fish were identified by labels on the holding tanks and test chambers.

F. Test Methods[(1)]

One nominal limit test concentration and a dilution water control were used in this study. The nominal limit test concentration of 120 mg/L H-28072 (not adjusted for 82.6% purity during preparation) was chosen for the definitive limit test based on the results of a preliminary rangefinding study.

Test chambers were stainless steel aquaria [30 (length) × 30 (width) × 30 (height) cm] which held approximately 20 L of test solution (26-L maximum volume; 22.5-cm test solution depth). One dilution water control test chamber and 3 replicate limit test concentration chambers with 10 fish in each chamber were used for testing (total of 10 fish in the control and 30 fish in the limit test concentration). Each chamber was covered with a glass plate to prevent fish from escaping. Random numbers were used to assign the position of test chambers in the waterbath.

Rainbow trout used in this study were not fed approximately 51 hours prior to nor during the test, and were assigned to the test chambers using random numbers. Addition of fish to the test solutions was initiated about 35 minutes after mixing of the test solutions was completed. Mortality and behavioral observations were made at test start, every 24 hours thereafter, and at test end. Dead fish were removed from the test chambers during daily observations. Criteria for death were the absence of opercular movement and lack of reaction to gentle prodding. At test conclusion, all surviving fish were humanely sacrificed using buffered MS222 (3-aminobenzoic acid ethyl ester).

A recirculating waterbath was used to maintain mean temperature in the test chambers during the 96-hour test at approximately 11.8°C with a range of 11.7 to 12.4°C (Table 5). In addition, a continuously-recording thermometer was used to check temperature variations in the waterbath. A photoperiod of 16 hours light (approximately 189-357 Lux) and 8 hours darkness was employed, which included 30 minutes of transitional light (52-136 Lux) preceding and following the 16-hour light interval.

Dissolved oxygen concentration, pH, and temperature were measured in all replicates of the dilution water control and limit test concentration. These measurements were taken before fish were added at test start, every 24 hours thereafter, and at test end. Total alkalinity, EDTA hardness, and conductivity of the dilution water control and limit test concentration were measured on samples collected at the beginning of the test. Test solutions were not aerated during the test and were disposed of in an appropriate manner at test end.

G. Sample Preparation and Chemical Analysis

1. Sample Collection

A full description of sample preparation and chemical analysis is presented in Appendix B.

Samples and back-up samples from test solutions containing H-28072 at a nominal concentration of 120 mg/L and the blank control were transported on ice from DuPont Haskell to the analytical laboratory for concentration verification on test days 0 and 4.

Concentrations of H-28072 in test solution samples were measured by high performance liquid chromatography with detection by mass spectrometry (LC/MS/MS).

RESULTS AND DISCUSSION

A. In-Life Data

1. Definitive Study

The nominal limit test concentration (not adjusted for 82.6% purity during preparation) for the definitive study was 120 mg/L. A dilution water control was used in this study. No H-28072 was detected in the dilution water control. The mean, measured concentration of H-28072 was 96.9 mg/L and was within 80-120% of the nominal limit test concentration.

Dilution water quality was acceptable based on OECD[(1)] dilution water criteria. Based on the most recent semi-annual dilution water analysis (Table 1), contaminant concentrations were below concentrations that could be expected to affect the integrity of a study. All chemical and physical parameters for the definitive test (Tables 2 - 5) were within expected ranges. Total alkalinity, EDTA hardness, and conductivity of the dilution water control and limit test concentration at test start ranged from 51 to 53 mg/L as $CaCO_3$, 126 to 132 mg/L as $CaCO_3$, and 255 to 280 µmhos/cm, respectively. During the test, dissolved oxygen concentrations ranged from 7.4 to 9.7 mg/L, pH ranged from 7.2 to 7.7, and mean temperature was 11.8°C with a range of 11.7 to 12.4°C.

At test conclusion, fish from the dilution water control ranged from 4.3 to 4.9 cm in standard length (mean 4.47 cm) and 0.941 to 1.493 g in wet weight, blotted dry (mean 1.068 g). Standard length of the longest fish was not more than twice the length of the shortest fish in the control. Loading in the water control was 0.534 g/L at test conclusion.

Data on daily mortality and sublethal effects are presented in Tables 6 and 7, respectively. No mortality or sublethal effects were seen in the dilution water control or at the nominal 120 mg/L (96.9 mg/L mean, measured) H-28072 concentration at the end of 96 hours. The 96-hour LC_{50}, based on the nominal H-28072 limit test concentration and mortality, was greater than 120 mg/L.

CONCLUSION

H-28072 was assessed for acute toxicity to unfed fingerling rainbow trout, *Oncorhynchus mykiss*, in an unaerated, static, acute, 96-hour limit test. The 96-hour LC_{50}, based on the nominal limit test concentrations of H-28072 and mortality, was greater than 120 mg/L.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at DuPont Haskell, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.

REFERENCES

1. Organisation for Economic Co-Operation and Development (OECD). Guideline for the Testing of Chemicals: 203, 17 July 1992.

TABLES

Table 1
Chemical Characteristics of DuPont Haskell Well Water[a]

Parameter	MDL[b]	Analytical Value	Parameter	MDL[b]	Analytical Value
BOD, mg/L	3.1	ND[c]	Lead, mg/L	0.0069	ND
COD, mg/L	2.6	ND	Magnesium, mg/L	0.0322	3.82
DOC, mg/L	1.0	ND	Manganese, mg/L	0.00036	0.007
TOC, mg/L	1.0	ND	MBAS/LAS, mg/L	0.035	ND
Total Kjeldahl N, mg/L	0.5	ND	Mercury, mg/L	0.000056	ND
Ammonia N, mg/L	0.03	ND	Nickel, mg/L	0.0056	ND
Turbidity, NTU	0.09	ND	Nitrite, mg/L	0.015	ND
Phenolics, mg/L	0.015	ND	Nitrate, mg/L	0.04	ND
Color, apparent Co/Pt[d]	5.0	ND	Ortho-phosphate, mg/L	0.01	ND
Solids			Potassium, mg/L	0.0503	3.94
total suspended, mg/L	1.5	ND	Selenium, mg/L	0.0005	ND
Aluminum, mg/L	0.0802	ND	Silver, mg/L	0.000023	ND
Antimony, mg/L	0.0097	ND	Sodium, mg/L	0.433	8.15
Arsenic, mg/L	0.00067	ND	Sulfate, mg/L	0.3	6.9
Beryllium, mg/L	0.00094	ND	Sulfide, mg/L	0.054	ND
Boron, mg/L	0.0094	0.0212 J[e]	Zinc, mg/L	0.0081	ND
Bromide, mg/L	0.4	ND	Ca/Mg	NA[f]	11.81
Cadmium, mg/L	0.000099	ND	Na/K	NA	2.07
Calcium, mg/L	0.104	45.1	Volatile priority		
Chloride, mg/L	4.0	66.5	pollutants, µg/L	0.5-40	ND
Chlorine, residual, mg/L	0.04	0.040 J	Acid extractable		
Chromium, mg/L	0.00026	0.00041 J	priority pollutants, µg/L	0.9-19	ND
Cobalt, mg/L	0.0021	ND	Base/neutral		
Copper, mg/L	0.0002	ND	priority pollutants, µg/L	0.9-19	ND
Cyanide, mg/L	0.005	ND	Pesticides/PCBs, µg/L	0.0019-0.28	ND
Iron, mg/L	0.0522	ND	Organophosphate		
Fluoride, mg/L	0.08	0.32	pesticides, µg/L	0.47-0.95	ND

[a] Sample analyses performed at Lancaster Laboratories, Lancaster, Pennsylvania, date of sample collection 11 May 2007 unless indicated otherwise.
[b] MDL = method detection limit, [c] ND indicates not detected at the MDL, [d] Units based on cobalt/platinum reference, [e] A "J" follows analytical values which were greater than the MDL but less than the limit of quantitation, [f] NA = not applicable.

Table 2
Water Chemistry of the Dilution Water Control and Limit Test Concentration at Test Start

Nominal H-28072 Concentration (mg/L)	Total Alkalinity (mg/L as $CaCO_3$)	EDTA Hardness (mg/L as $CaCO_3$)	Conductivity (μmhos/cm)
Dilution Water Control	51	132	255
120	53	126	280

Table 3
Dissolved Oxygen Concentration (mg/L)[Ψ] of H-28072 Test Solutions

Nominal H-28072 Concentration (mg/L)	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
Dilution Water Control	9.6	8.0	7.7	7.7	7.7
120 A†	9.7	8.2	7.7	8.0	8.0
120 B†	9.6	8.1	7.6	7.9	8.0
120 C†	9.7	7.9	7.4	7.7	7.7

Ψ The theoretical dissolved oxygen concentrations at 100% saturation is 9.1 mg/L at 20°C.
† Replicate test chambers contained 10 fish each at test start.

Table 4
pH of H-28072 Test Solutions

Nominal H-28072 Concentration (mg/L)	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
Dilution Water Control	7.3	7.3	7.2	7.2	7.2
120 A†	7.7	7.5	7.4	7.3	7.2
120 B†	7.7	7.5	7.4	7.3	7.3
120 C†	7.7	7.5	7.3	7.2	7.2

† Replicate test chambers contained 10 fish each at test start.

Table 5
Temperature (°C) of H-28072 Test Solutions

Nominal H-28072 Concentration (mg/L)	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours
Dilution Water Control	12.3	11.7	11.8	11.7	11.7
120 A†	12.3	11.7	11.8	11.7	11.7
120 B†	12.4	11.7	11.7	11.7	11.7
120 C†	12.4	11.7	11.7	11.7	11.7

† Replicate test chambers contained 10 fish each at test start.

Table 6
Mortality of Rainbow Trout, *Oncorhynchus mykiss*, in an Unaerated Static, Acute, 96-Hour Limit Test with H-28072

Nominal H-28072 Concentration (mg/L)	Number Dead / Number at Study Start			
	24 Hours	48 Hours	72 Hours	96 Hours
Dilution Water Control	0/10	0/10	0/10	0/10
120 A†	0/10	0/10	0/10	0/10
120 B†	0/10	0/10	0/10	0/10
120 C†	0/10	0/10	0/10	0/10

† Replicate test chambers contained 10 fish each at test start.

Table 7
Sublethal Effects of Rainbow Trout, *Oncorhynchus mykiss*, in an Unaerated Static, Acute, 96-Hour Limit Test with H-28072

Nominal H-28072 Concentration (mg/L)	Number with Effects / Number Alive			
	24 Hours	48 Hours	72 Hours	96 Hours
Dilution Water Control	0/10	0/10	0/10	0/10
120 A†	0/10	0/10	0/10	0/10
120 B†	0/10	0/10	0/10	0/10
120 C†	0/10	0/10	0/10	0/10

† Replicate test chambers contained 10 fish each at test start.

APPENDICES

Appendix A
Certificate of Analysis

E. I. du Pont de Nemours and Company
Wilmington. DE 19898
USA

CERTIFICATE OF ANALYSIS

This Certificate of Analysis fulfills the requirement for characterization of a test substance prior to a study subject to GLP regulations. It documents the identity and content of the test substance. This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 792).

Haskell Code Number	H-28072
Common Name	HFPO Dimer Acid Ammonium Salt
Purity Percent	82.6%
Other Components	Water – 13.9% Ammonium (excess) – 3.5%
Date of Analysis	July 19, 2007
Recommended reanalysis interval	1 year
Instructions for storage	NRT&H
Reference	DuPont-23285
Analysis performed at	E. I. DuPont de Nemours and Company DuPont Haskell Laboratories Newark, Delaware USA

Peter A. Bloxham, Ph.D.
Analyst's Name


Analyst's signature

23-JUL-2007
Date

Revision #1
July 20, 2007

Appendix B
Analytical Report

Test Solutions Analyses:

H-28072: Static, Acute, 96-Hour Limit Test with Rainbow Trout, *Oncorhynchus mykiss*

AUTHOR: Karen M. L'Empereur, Ph.D.

ANALYTICAL STUDY COMPLETED ON: 19-July-2007
REPORT REVISED ON: 17-August-2007

PERFORMING LABORATORY: Critical Path Services (CPS)
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SPONSOR: E.I. du Pont de Nemours and Company
Wilmington, DE 19898

CPS PROJECT NUMBER: 07-CPS-021

SPONSOR PROJECT NUMBER DuPont-22830, Rev. 1

WORK REQUEST NUMBER: 17199

SERVICE CODE NUMBER: 228

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This analytical phase of this study was conducted in compliance with U.S. EPA TSCA (40 CFR Part 792) Good Laboratory Practice Standards, which are compatible with the OECD and MAFF Japan Good Laboratory Practice Standards.

CPS
Principal Investigator: Karen M. L'Empereur Date: 17-Aug-07
Karen M. L'Empereur, Ph.D.
Critical Path Services

QUALITY ASSURANCE STATEMENT

This study was inspected/audited by Quality Assurance according to CPS Standard Operating Procedures and EPA's Good Laboratory Practice Standards (40 CFR Part 792) and all findings were reported to the Study Director and Management. It was concluded that the final report accurately reflects the raw data for this study.

Phase Audited	Date of QAU Inspection	Date Reported to Study Director	Date Reported to Management
Standard Preparation	04-June-2007	04-June-2007	04-June-2007
Study Records, Final Report	17-July-2007	18-July-2007	18-July-2007
Revised Final Report	17-August-2007	17-August-2007	17-August-2007

CPS
Quality Assurance Auditor: _____ Date: 17 August 2007

Susan C. Nicastro
Critical Path Services

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from the analytical phase of this study.

CPS
Principal Investigator: Karen M. L'Empereur Date: 17-Aug-07
Karen M. L'Empereur, Ph.D.
Critical Path Services

CPS Management: Julie E. Eble Date: 17 August 07
Julie E. Eble, Ph.D.
Critical Path Services
Laboratory Director

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SUMMARY

Samples from the limit test solutions containing H-28072 at a nominal concentration of 120 mg/L and the dilution water control were submitted for concentration verification on test day 0. Samples from 3 replicate chambers of the limit test concentration and the dilution water control chamber were submitted at test end (day 4).

Concentrations of H-28072 in test solution samples were measured by high performance liquid chromatography with detection by mass spectrometry (LC/MS/MS).

The mean, measured value of H-28072 in the 3 replicate chambers of the limit test concentration was 97.7% of the targeted nominal test concentrations adjusted for test substance purity of 82.6%.

Control solutions contained no detectable concentrations of H-28072.

The report has been revised to reflect the change in the assigned purity of the H-28072 test substance from 81.4% to 82.6%. This change does not affect the conclusions of the study.

MATERIALS AND METHODS

A. Sample Preparation and Chemical Analysis

1. Sample Collection and Treatment

One sample plus a back-up sample of the test solution was received from each replicate test chamber at the limit test concentration and from the dilution water control on days 0 and 4 of the definitive study. Samples and back-up samples were transported on ice to the analytical laboratory, and were stored refrigerated upon receipt and when not in use.

The samples, including controls, were diluted 2000x, with a solution of HPLC grade water/acetonitrile, 50/50, v/v, before analysis. Dilution of the samples was necessary due the sensitivity of the detector to H-28072.

Concentrations of H-28072 were measured by high performance liquid chromatography with detection by mass spectrometry (LC/MS/MS) in samples that were stored refrigerated and analyzed within 2 days of sample receipt.

2. Instrument and Conditions

HPLC Instrument:	Agilent Model 1200
MS Instrument:	Applied Biosystems API 4000
Software:	Analyst 1.4.1

LC Parameters:

Column:	Phenomenex Luna C8; 150 x 2.0 mm, 5.0 µm
Mobile Phase:	Premix of water/acetonitrile, 50/50, v/v, with 0.15% acetic acid and 0.15% triethylamine
Flow Rate:	0.400 mL/min
Column Temperature:	30 °C
Injection Volume:	3.0 µL

MS Parameters:

Polarity (+/-)	Q1 (m/z)	Q2 (m/z)	Dwell (msecs)	CUR (psi)	GS1 (psi)	GS2 (psi)	TEM (°C)	iHe	IS (V)	DP (V)	EP (V)	CE (V)	CXP (V)
-	329.00	285.00	100	40	11	50	400	on	-4500	-20	-10	-10	-5

3. Quantitation

A primary stock solution of the test compound, H-28072 (purity 82.6%), was made by dissolving the standard in water. Appropriate aliquots of the primary stock solution were diluted with dilution water (HLWW) to prepare a secondary stock solution. On each day of analysis the sccondary stock solution was diluted with a solution of water/acetonitrile, 50/50, v/v to give calibration standards with concentrations that bracketed the concentrations of the diluted test solutions. Duplicate injections of test and calibration standard solutions were made and peak areas were determined electronically.

The calibration standard curve was generated by regression analysis using the chromatographic peak areas of the calibration standard solutions. Data for test solutions were compared to the calibration standard curve to determine concentrations of H-28072. The limit of detection (LOD) was determined by calculating the average noise level in chromatograms of the dilution water control solutions and comparing them to the signal of a calibration standard of known concentration. Two chromatograms were examined for noise-related peaks near the retention time of the analyte. The LOD was calculated as 3 times the concentration equivalent of the mean noise level. The limit of quantitation (LOQ) was defined as the greater of 10 times concentration equivalent of the mean noise level or the lowest standard concentration.

RESULTS AND DISCUSSION

A. Analytical Report

1. Chromatographic Results

H-28072 eluted as a well-resolved chromatographic peak with a retention time of approximately 2.1 minutes. A typical calibration standard curve is shown in Figure 1. Representative chromatograms of a calibration standard solution, a dilution water control solution sample, and a test solution sample are presented in Figures 2 to 4, respectively.

The LOD and LOQ were determined to be 0.0001 µg/L and 25.9 µg/L, respectively.

2. Test Solution Results

The mean, measured value of H-28072 in the 3 replicate chambers of the limit test concentration was 97.7% of the targeted nominal test concentration adjusted for test substance purity of 82.6% (Table 1). All measured values of H-28072 were within 1.5X of the lowest value for all samples within the concentration. These data indicate that the H-28072 limit test concentration was maintained at acceptable levels throughout the definitive test.

Control solutions contained no detectable concentrations of H-28072 (Table 1).

TABLE 1

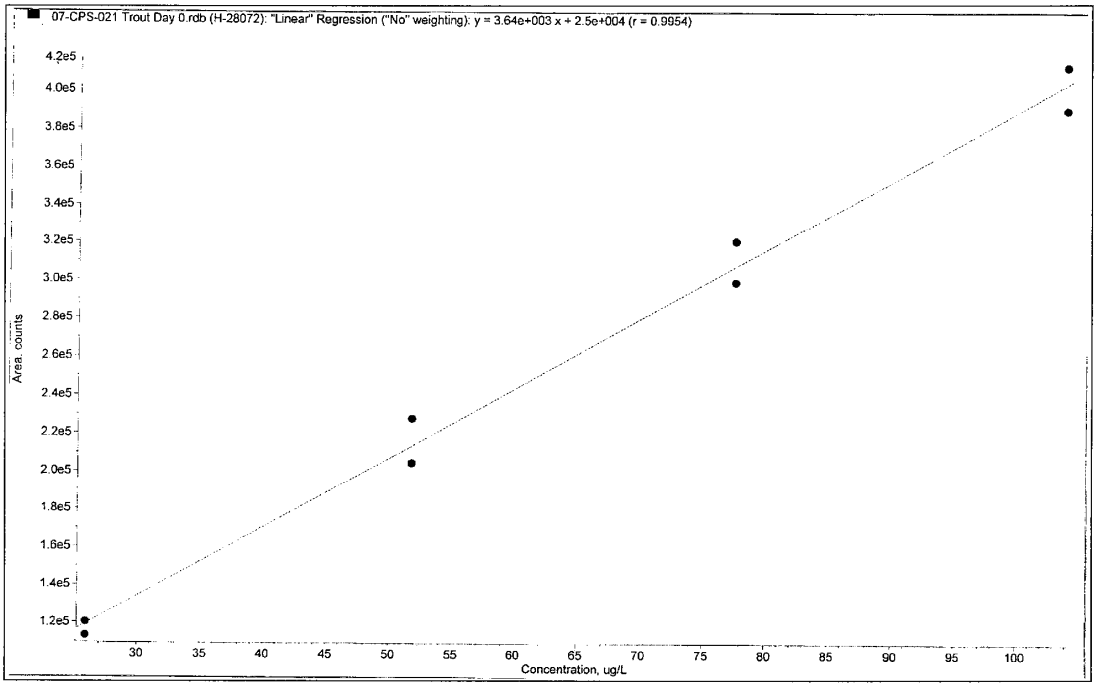
MEASURED CONCENTRATIONS OF H-28072 IN TEST SOLUTIONS

Nominal H-28072 Concentration (mg/L)	Nominal H-28072 Concentration, Adjusted for Purity (mg/L)[a]	Measured H-28072 Concentration (mg/L) Day 0	Measured H-28072 Concentration (mg/L) Day 4	Mean, Measured Concentration (mg/L)[b]	Percent Recovery (%)[c]
Water Control	0.0	ND[e]	ND	---	---
120 A[d]	99.1	97.2	95.6	96.9	97.7
120 B[d]	99.1	98.1	92.5		
120 C[d]	99.1	102	95.7		

a Nominal H-28072 concentrations adjusted for 82.6% purity.
b Mean, measured H-28072 concentration was calculated as the average of all measured values at the test concentration.
c Based on nominal concentration adjusted for purity.
d Replicate identification.
e ND denotes not detected. The limit of detection for H-28072 was calculated as 0.0001 µg/L.

FIGURE 1

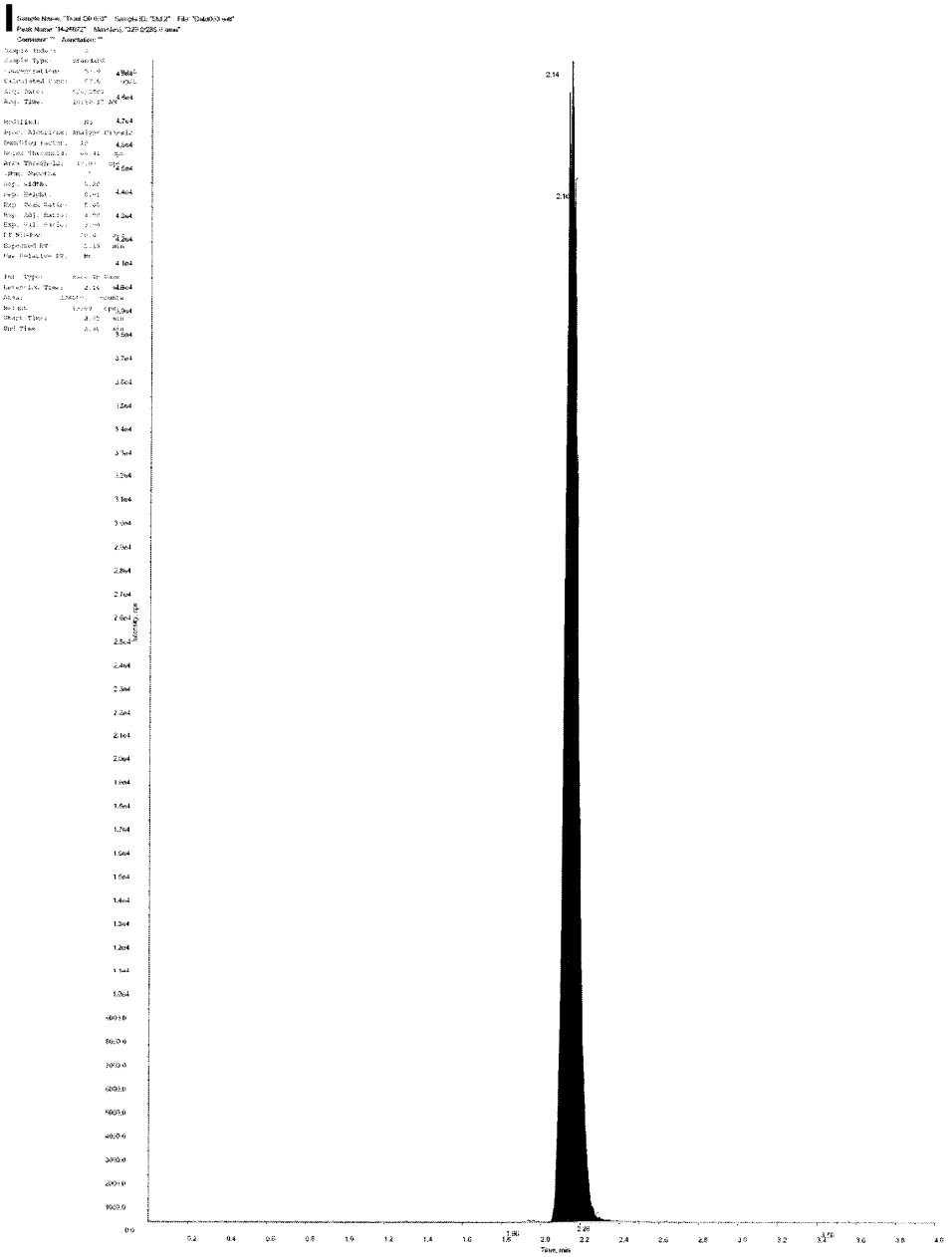
REPRESENTATIVE ANALYTICAL CALIBRATION STANDARD CURVE FOR H-28072



Concentration of H-28072 in µg/L

FIGURE 2

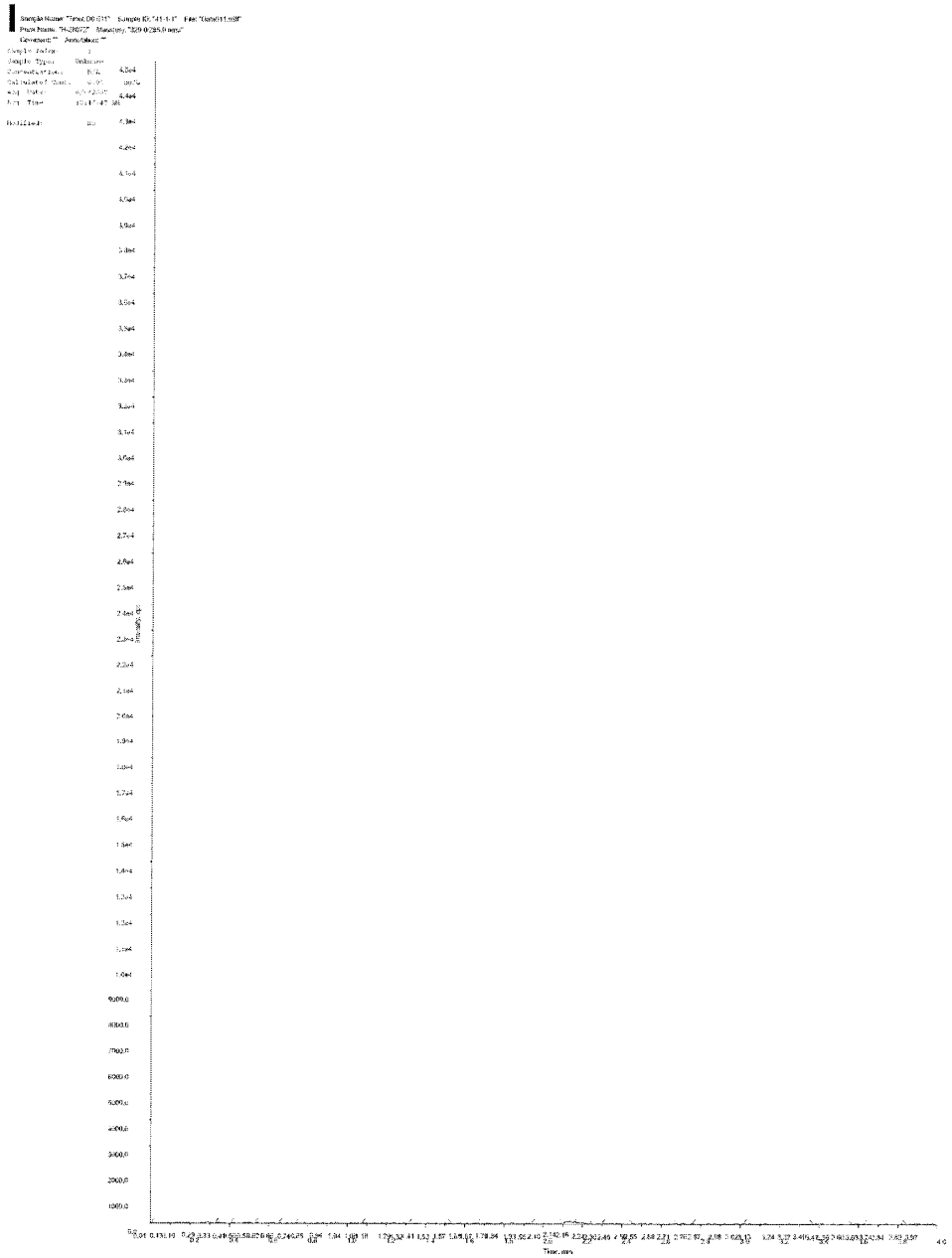
REPRESENTATIVE CHROMATOGRAM OF A CALIBRATION STANDARD SOLUTION



H-28072 elutes at a retention time of approximately 2.16 minutes. The calibration standard solution contains H-28072 at a concentration of 51.8 µg/L.

FIGURE 3

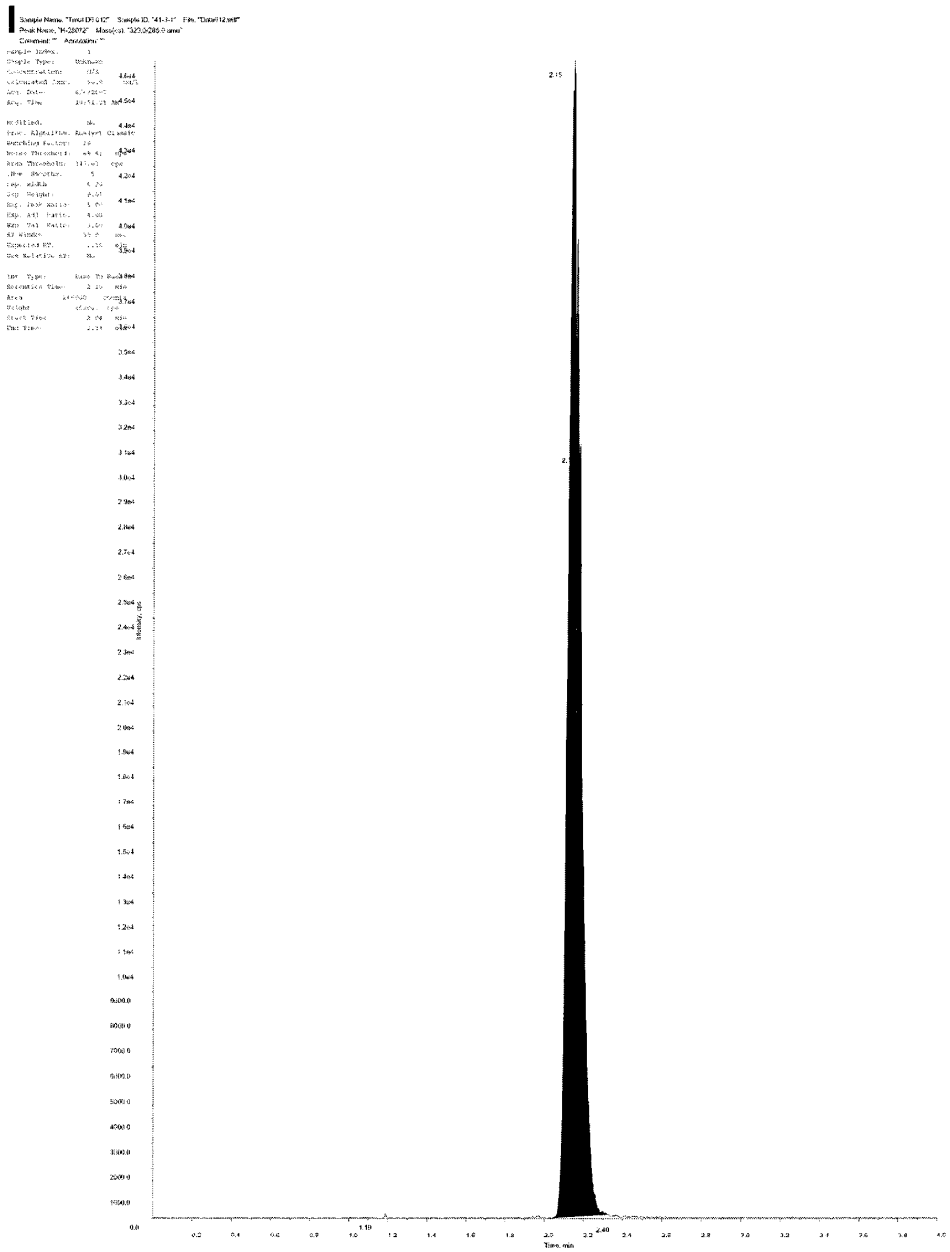
REPRESENTATIVE CHROMATOGRAM OF A DILUTION WATER CONTROL SOLUTION



H-28072 would elute at a retention time of approximately 2.16 minutes.

FIGURE 4

REPRESENTATIVE CHROMATOGRAM OF A H-28072 TEST SOLUTION



H-28072 elutes at a retention time of approximately 2.15 minutes. The test solution sample contains H-28072 at a nominal concentration of 60 µg/L. The sample was diluted 2000x with acetonitrile/water, 50/50, v/v, prior to analysis.